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REVIEW

C-reactive Protein (CRP) in Animals: Its Chemical Properties and Biological Functions

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ABSTRACT—C-reactive protein (CRP), which was found in the sera of pneumococci in 1930 and has been used as a marker protein for inflammation in clinical laboratories, has been isolated as well from the sera of fishes and body fluids of invertebrates. Taking it into consideration that the evolutionary origin of this protein may be early enough, the CRP is supposed to act an important role. Recent studies on CRP have revealed the biological functions of this protein. For example, CRP and macrophages make up an amplification mechanism in primary stage of immune response. On the other hand, it was revealed that the serum level of CRP was controlled by not only interleukin(s) but also sex hormone(s).

INTRODUCTION

The animals, in their serum, have *les jumellaux* that function in the first stage of defence system, being neither immunoglobulins nor proteases such as complements. They are C-reactive protein (CRP) and serum amyloid P component (SAP). Both have similar physicochemical properties and electronmicroscopically a cyclic pentamer form. Although CRP has been used as a marker protein for inflammation in clinical laboratories, its evolutionary conservation suggests an important role of this protein. Recent studies have been revealed immunological functions of CRP, for example activation of marcophages and tumoricidal activity. Furthermore, CRP solubilizes endogenous substances derived from damaged cells such as chromatin cooperating with complements.

In this article, I would like to summerize the chemistry of CRP and discuss its biological and immunological functions, focusing the experimental results with rats and japanese eel (*Anguilla japonica*) in our laboratory.

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SHORT HISTORY OF STUDIES ON CRP

In 1930, Tillett and Francis observed that sera of patients during acute febrile illnesses produced a precipitation with a component in the extract of pneumococcus, first designated Fraction C and later C-polysaccharide [1]. The substance responsible for this reaction was termed "C-precipitin". In 1941, Abernathy and Avery [2] showed that the substance bound to C-polysaccharide in the membrane of *Streptococcus pneumoniae* in the presence of calcium ion, and the reaction was inhibited by phosphorylcholine (PC). The authors designated the substance as "C-reactive protein". In 1947, McCarty succeeded in crystalization of CRP and raising specific antibody in rabbit [3]. Since 1947, the serum level of CRP has been measured by the immunoassay, for example single radial immuodiffusion and/or enzyme-immunoassay using specific antibody [4–6]. Gotshclich and Edelman observed that CRP consisted of 24 kDa of five identical subunits associated hydrophobically [7]. In 1977, an electronmicroscopic observation by Osmand *et al.* demonstrated the shape of CRP being cyclic pentamer [8]. The authors proposed in their report that CRP and

SAP were termed "pentraxin".

The interaction of CRP and complements has been studied since 1974. Once CRP has complexed with PC residues of phospholipids such as lecithin and sphingomyelin, the complex activates the classical pathway [9]. In 1980, DiCamelli *et al.* reported the binding of CRP to polycations [10]. The *in vitro* experiments clarified that the CRP bound to nuclear proteins, such as histone and protamine, more strongly than PC [11–13]. CRP seems to relate to the solubilization of chromatin following complement activation by CRP-chromatin complex [14].

The tumoricidal effect of human CRP has been studied since 1982. It was observed that human CRP inhibited metastases of melanoma to lung in mouse [15–17]. Several reports described that tumor cells were killed by human CRP, having mouse macrophages to produce superoxide-anion *in vitro* [18–21].

The CRPs are found in the sera of some vertebrates including fishes, for example rabbit [22, 23], bovine [24], dog [25], goat [26], plaice (*Pleuronectes platessa*) a marine teleost [27], rainbow trout (*Salmo gairdneri*) [28, 29], and dogfish (*Mustelus canis*) [30]. Almost phylogenic studies on CRP have dealed with its purification and physicochemical analysis, but there are few studies on its biological activity. However, in 1981, a CRP was isolated from the body fluid of horseshoe crab (*Limulus polyphemus*) and its lectin activity was studied by Robey and Liu [31]. In our laboratory, a CRP was isolated from the serum of japanese eel (*Anguilla japonica*) [32] and the CRP analogue from the serum of female whiteedged rockfish (*Sebastes taczanowskii*), a typical viviparous marine teleost [33]. It was demonstrated that these molecules have also lectin activity.

A topological analysis of CRP from hoseshoe crab, human, rabbit and human SAP indicated that these proteins may originate from the same ancestral gene [34].

Since 1982, the epitopes of human CRP have been analysed by using mouse monoclonal antibody by Kilpatrick *et al.* [35] and Roux *et al.* [36]. CRP has at least two different epitopes; the one is locted near or at the PC binding site, the conformation of which is changed by chelation with calcium ion. In 1987, Potempa *et al.* [37] observed that the subunit of CRP, termed "neo-CRP", had a different antigenecity from the native CRP molecule. However, there is few reports on the analysis of epitope(s) and biological significance of "neo-CRP" in other animals [38].

CHEMICAL APPROACH TO CRP

Purification of CRP

The ligand specificity of CRP and SAP in the presence of calcium ion differes completely [39]. The CRP specifically binds to PC and the SAP to agarose, respectively. The CRP can be specifically precipitated by being mixed with PC or C-polysaccharide in the presence of calcium ion. CRP in the serum of rat or eel was purified by the lecithin precipitation method described by Hokama *et al.* [40]. In brief, serum was mixed with soy-bean lecithin and then the mixture was dialyzed against 0.01 M $CaCl_2$. The precipitate was dissolved in sodium citrate buffer, pH 7.0 and then chloroform was add to the mixture. After centrifugation, the upper aqueous layer was dialyzed against $CaCl_2$. The precipitate was again dissolved in sodium citrate buffer pH 7.0. Further purification was carried out chromatography on DEAE-Sephacel and gel filtration on S-300. The purification procedure was previously reported in detail [32, 41]. Recently, a new method for purification of CRP in rat serum has been developed in our laboratory. Rat sera were brought to between 209 g/l and 409 g/l of ammonium sulfate at pH 7.2. The precipitate was extensively dialysed against 10 mM Tris-HCl buffer, pH 8.0 containing 0.14 M NaCl and 10 mM $CaCl_2$ (Buffer A) and then applied to a column of Sepharose 4B loaded with 100 mg of protamine (derived from salmon sperm, grade X, Sigma). Washing the column with 10 mM Tris-HCl buffer, pH 8.0 containing 1.14 M NaCl, bound CRP was eluted with 10 mM Tris-HCl buffer, pH 8.0 containing 1.14 M NaCl and 0.5 M arginine-HCl. Eluted fraction was dialysed against Buffer A and then applied to a column of Arginine-Sepharose 4B (Pharmacia). The CRP was eluted with 50 mM sodium citrate buffer pH 7.0 containing 0.2 M NaCl. Further purification was

carried out by gel filtration on S-300 [42]. The recovery by the new method was higher than that by lecithin-precipitation. On the other hand, PC- or C-polysaccharide-Sepharose affinity column chromatographies are widely used for the purification of CRP [43, 44]. However, not only the coupling of PC with Sepharose is rather difficult, but also a sufficient amount C-polysaccharide for an affinity column is not easy to obtain [45]. We tried to purify CRP from rat serum using the PC-Sepharose, however, many contaminating proteins of a similar characteristics as CRP appeared. De Beer *et al.* succeeded in purification of rat CRP by C-polysaccharide-Sepharose affinity chromatography [46]. Using the PC-Sepharose, the CRPs were actually purified from sera of human, rabbit, goat [47], white-edged rockfish [33] and eel in our laboratory. Figure 1 shows a cellulose-acetate membrane electrogram of purified human and eel CRP. In addition, using Sepharose coupled with mouse monoclonal antibody, human CRP was highly purified without destroying its hydrophobic association [48].

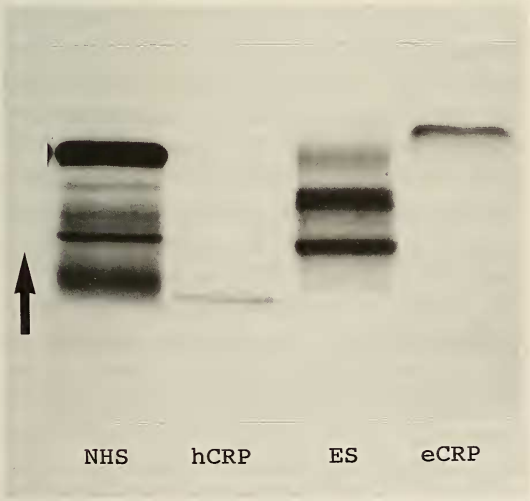

Fig. 1. Cellulose-acetate membrane electrophoresis of human and eel CRP. NHS, normal human serum; hCRP, human CRP; ES, eel serum; eCRP, eel CRP. Anode is top.

Physicochemical chracteristics of CRP

The molecular weight of rat CRP was 165 kDa determined by gel filtration and that of its subunits

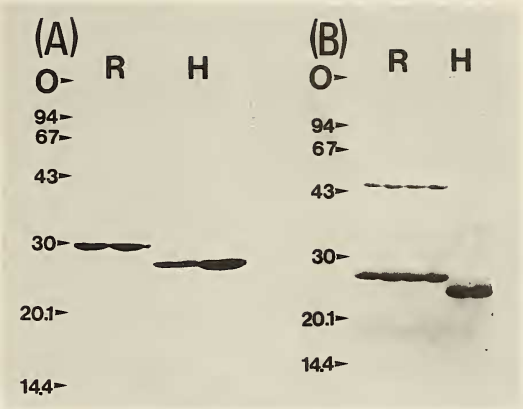

Fig. 2. SDS-PAGE of CRP under reducing (A) with 2-mercaptoethanol and non-reducing (B) conditions. R and H represent rat and human CRP, respectively. Marker proteins are as follows: rabbit muscle phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), bovine erythrocyte carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and bovine milk α-lactalbumin (14.4 kDa).O, origin. Each CRP sample (20 μg) were run in plural lanes.

was 29 kDa estimated by SDS-PAGE (Fig. 2). On SDS-PAGE, without reducing by 2-mercaptoethanol (2ME), CRP appeared as two bands, 27 kDa and 53.5 kDa. The results indicate that the rat CRP has a disulfide bond between in its subunits [41, 46]. By gel filtration and SDS-PAGE, eel CRP was found to consist of 24 kDa five identical subunits [32]. No disulfide bonds among the subunits was detected in the eel CRP molecule as well as CRPs of human, rabbit and goat.

The electrophoretical mobilities of CRPs differed each other among the animal species. In electrophoresis at pH 8.6, rat CRP [41] and eel CRP moved near the region of albumin. In contrast human CRP moved to the region of gamma globulin as shown in Fig. 1. The CRPs of rabbit and goat moved as slowly as the latter CRP.

The isoelectric points of rat CRP were 4.29, 4.22, 4.21 and 4.16 determined by the thin layer polyacrylamide isoelectricfocusing. In O'Farrell's two dimension electrophoresis [49], the first dimension being isoelectricfocusing in polyacrylamide gel containing 9.6 M urea and the second dimension being SDS-PAGE, rat CRP gave two spots of pH 5.3 and 5.4, each molecular weight

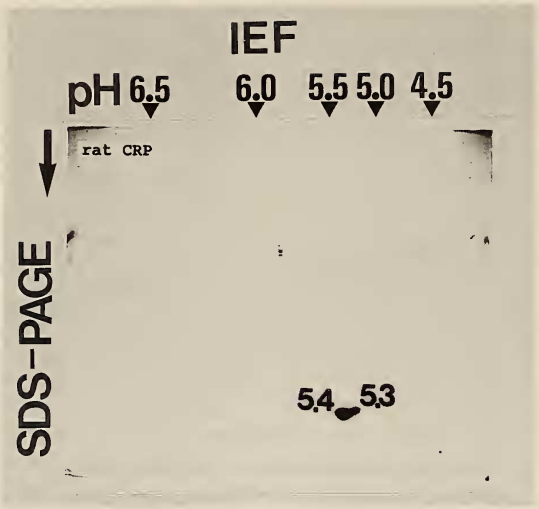

FIG. 3. O'Farrell's two dimension electrophoresis of rat CRP. The first dimension is isoelectricfocusing containing 9.6 M urea in polyacrylamide and the second is SDS-PAGE in gradient gel.

being 27 kDa (Fig. 3). Since rat CRP could move in a gel containing high concentration of urea, hydrophobic binding of CRP would disociate. The results suggest that the isoelectric points of the subunits of rat CRP were different from the native pentameric molecule.

AGGLUTINATING ACTIVITY OF CRP

CRPs of rat, eel and white-edged rockfish strongly agglutinate *Streptococcus pneumoniae* in the presence of calcium ion and the agglutinting activity is inhibited by PC or chelation of calcium ion (EDTA addition), as in the case of human CRP. However, bacteria having "Type 4" polysaccharide which does not contain PC residue were agglutinated by human CRP [50].

The CRP of horseshoe crab agglutinated horse red blood cells and the activity was inhibited sialic acid [31]. The result suggests that the CRPs of animals in lower classes may have a lectin activity. Actually, eel CRP strongly agglutinated rabbit red blood cells in the presence of calcium ion (Fig. 4). This agglutinating activity was inhibited by D-glucosamine and D-mannose [32]. A lectin purified from eel serum [51] agglutinated human H(O) type red blood cells via fucose residue [52]. The physicochemical and biological characteristics

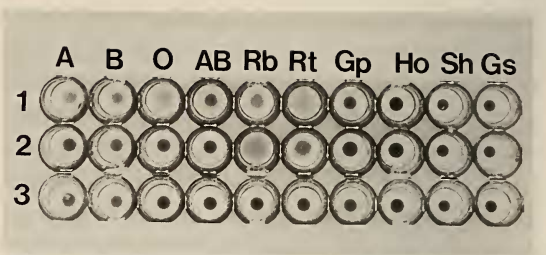

FIG. 4. Agglutinating activity of eel and human CRP in the presence of calcium ion (GVB^{++}). Lanes 1–3 are eel serum, eel CRP and human CRP, respectively. The final concentration of each CRP was 20 μg/ml. Abbreviations A, B, O, AB, human blood cell types; Rb, rabbit; Rt, rat; Gp, gunia pig; Ho, horse; Sh, sheep; Gs, goose red blood cells. Fresh blood cells were used for this experiment.

of the lectin completely differ from those of eel CRP. Furthermore, CRP purified from the serum of female white-edged rockfish also agglutinated rabbit red blood cells and the activity was inhibited by D-glucosamine and N-acethyl-D-galactosamine. In contrast, rat and human CRP could not agglutinate any red blood cells. Uhlenbruck *et al.* reported that human CRP formed a precipitin line with galactan in haemolymph of *Helix pomatia* and *Octopus vulgaris* in agarose gel containing calcium ion [53, 54]. Soelter and Uhlenbruck described that the anti-galctan activity of the human CRP was attributed to its anti-PC activity and not to the lectin activity [55]. However, it is possible to consider that the lectin-like activity remains in the human CRP. Fish CRPs belong to a "lectin family", but rat and other mammals' ones do not. The animals in low classes do not have active immunoglobulins, such as immunoglobulin G (IgG). Fish, for example chum salmon (*Oncorhynchus keta*) have only one class of immunoglobulin M (IgM) [56]. The results suggest that the CRP may possibly be one of the major defence substances in animals of lower classes. The role of CRP has been changed along the development of the defence system through the evolution of animals. Two lectins were purified from the plasma of ascidia *Didemnum candidum*, termed DCL-I and DCL-II. The N-terminal amino acid sequence of DCL-I showed up to six identities in a stretch of 19 residues with human CRP [57]. In addition, DCL-I cross-reacted in

TABLE 1. Biochemical Properties of CRPs

	human	rat	eel	
Molecular Weight	110	165	120	Gel filtration (S-300)
(kDa)	24	29	24	SDS-PAGE (+2ME)[a]
	24	27+53.5	24	SDS-PAGE (−2ME)
Shape	pentamer	pentamer	pentamer	
Mobility	γ	α	Albumin	pH 8.6, μ=0.05
Isoelectricpoint	6.4[b]	4.16−4.29	N.D.	
	N.D.	5.3+5.4	N.D.	O'Farrell's method[c]
Binding activity				
phosphorylcholine	Yes	Yes	Yes	
polycation	Yes	Yes	Yes	
nuclear protein	Yes	Yes	Yes	
sugars	galactan	no	D-glucosamine D-mannose	

N.D. not determiend.
[a] 2-mercaptoethanol
[b] Potempa *et al.* [91]
[c] O'Farrell [49]

enzyme-linked immunosorbent assays (ELISA) with antibodies made against human CRP. Although Sir M. Burnet long ago suggested that invertebrates lectins might be related to the early precursors of immunoglobulins found in vertebrates, little subsequent information has been obtained to support this hypothesis. Recent evidences of amino acid sequence, immunochemical cross-reaction and agglutination activity suggest that CRP seems to be ascribed to invertebrates lectins.

Both eel CRP and human CRP reacted with polycations in agarose gel [32]. Robey *et al.* [14] reported a precipitation reaction of rabbit CRP with nucleosome core particles in agarose gel, and suggested a possibility of CRP for acting as a scavenger of chromatin fragments from damaged cells. As eel has only undevelopoed immune net work, it has not known whether the autoimmune response seen in mammals actually occur in eel as well. That is a very interesting problem of CRP in phylogeny. The biochemical properties of CRPs are summerized in Table 1.

ANTIGENECITY OF CRP

Rat CRP does not react with antiserum raised against CRP of the other species (Oucterlony's method, Fig. 5). In addition, mouse monoclonal antibodies to human CRP recognizing two different epitopes [58] also did not react with rat CRP in ELISA system. However, the immunochemical cross-reactions were observed among the human, rabbit and goat CRPs. Maudsley and Pepys reported that immunochemical cross-reactions among CRPs of 30 kinds of animals including lower classes [59]. They observed no immunochemically cross-reaction between rat CRP

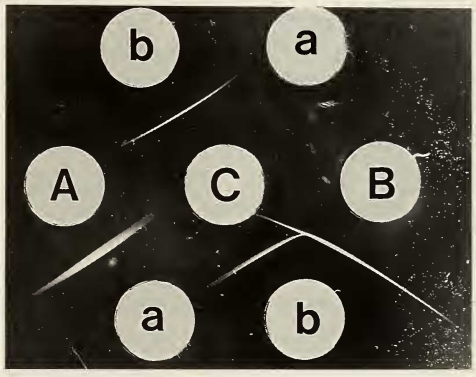

FIG. 5. Immunochemical cross-reactoin among the CRPs in agarose gel. A, rat CRP; B, human CRP; C, rabbit CRP; a, goat antiserum to rat CRP; b, goat antiserum to human CRP.

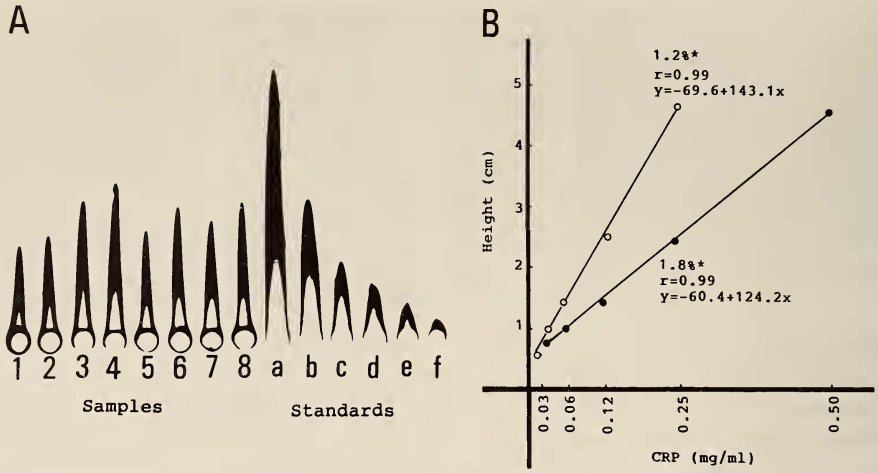

FIG. 6. Rocket immunoelectrophoresis of rat CRP (A) and the standard curve of rat CRP for rocket immunoelectrophoresis (B). The numbers (1–8) are samples of rat sera and a-f are standards of rat CRP. a, 0.402 mg/ml; b, 0.268 mg/ml; c, 0.134 mg/ml; d, 0.067 mg/ml; e; 0.05 mg/ml. In B, 1.2% and 1.8% represent the concentration of rabbit antiserum to rat CRP in agarose gel.

and CRPs of other 9 kinds of mammals. Although the chemical characteristics of rat CRP are different from those of human, Taylor *et al.* reported that 45 residues of carboxyl terminal of rat CRP had 71.7% similarity to that of human CRP [60, 61]. Three dimensional conformation of both CRPs seem to be so different each other as to yield a common antigenecity. On the other hand, an antibody to eel CRP formed a precipitin line with the CRP analogue of white-edged rockfish [32, 33]. The precipitin line is spur, indicating a partial identical antigenecity between them. However, there are no reports on the cross-reaction among neo-CRP (subunit of CRP) of various animals.

CRP AS A TYPICAL ACUTE PHASE REACTANT

Serum level of CRP

The serum level of rat CRP was measured by so-called "rocket immunoelectrophoresis" [62] as shown in Fig. 6, and by sandwich ELISA. CRP level of normal adult rat is approximately 0.5 mg/ml, being 100–5000 times higher than other animals, for example 100 ng/ml in human [4–6], 1.5 μg/ml in rabbit [63], 60 μg/ml in dog [25], 30 μg/ml in rainbow trout [29], 1 μg/ml in eel [32]. The concentration of CRP in the body fluid of the

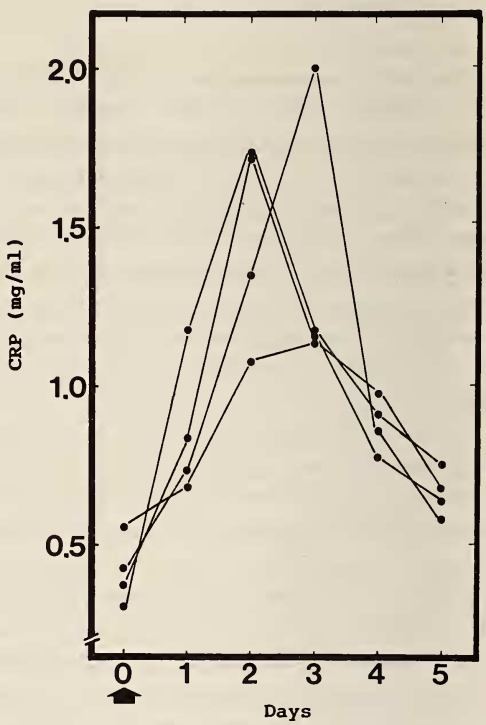

FIG. 7. Serum CRP levels in rats with chemically-induced inflammation. Four rats were injected intramuscularly with 1 ml of turpentine-oil on Day 0 (indicated by the arrow) and the serum levels were followed up to Day 5 by rocket immunoelectrophoresis.

horseshoe crab was 40% of total protein [31]. The differences of serum level of CRP in various species may be caused by their adptation to the environment.

CRP in chemical inflammation

The serum level of CRP immediately increases after destruction of tissues, for example bacterial infection or intoxication of turpentine-oil injection. Although this increase in inflammation was 1000-times in human and rabbit [39, 63], rat CRP elevated approximately 3–4 times after the injection of turpentine-oil (Fig. 7). Rats have also plural acute phase reactants in the serum as well as human. In rat, the α_1-acid glycoprotein (AGP) is a major acute phase reactant [64]. By immunohistochemical method, CRP was demonstrated in the cytoplasm of hepatocytes but not in macrophages in liver, named specially "Kupffer cells" (Fig. 8A) and thymus and spleen. It has been evidenced by *in vitro* experiment that CRP localizes in hepatocytes but not lymphoid tissues. In rat, CRP was detected in the medium of primary culture of hepatocytes but not in that of lymphocytes [65].

CRP was immunohistochemically detected on the surface of injured tissues (Fig. 8C) and the surface of white blood cells infiltrated to the site of the inflammation (Fig. 8D). It is interesting whether lymphocytes have a specific receptor for CRP or not. Zeller *et al.* reported that human monocytes had CRP binding site distinct from IgG receptor [66, 67]. According to Tseng and Mortensen, CRP binds to fibronectin in the presence of calcium [68]. Therefore, it is considered that CRP binds to white blood cells through the fibronectin on the cells. It is interesting to investigate the mechanism of binding of CRP and lymphocytes.

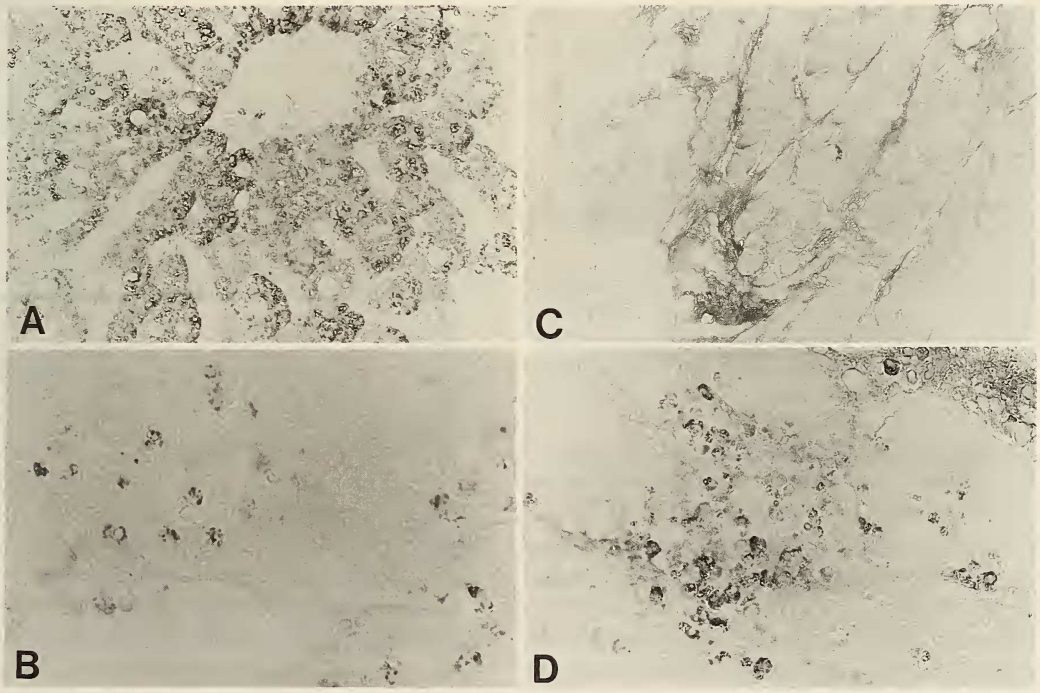

FIG. 8. Immunohistochemical localization of CRP. (A) in hepatocytes of a rat injected with turpentine-oil 2 days in advance (×660), (B) in hepatocytes of a rat injected with CCl_4 (8 hr after injection), ×1000, (C, D) muscle tissues 2 days after the turpentine-oil injection, C; ×330 and D; ×500. CRP deposits were found injured muscle and in intravenous space between mucle fibers (C) and in cytoplasm of infiltrated white blood cells (D). Specificity of antibody in rat CRP was examined by immunoelectrophoresis [41]. The avidin-biothin complex method was employed for the immunostaining. Note CRP was not detected in the normal muscle, liver macrophages (Kupffer cells) or red blood cells.

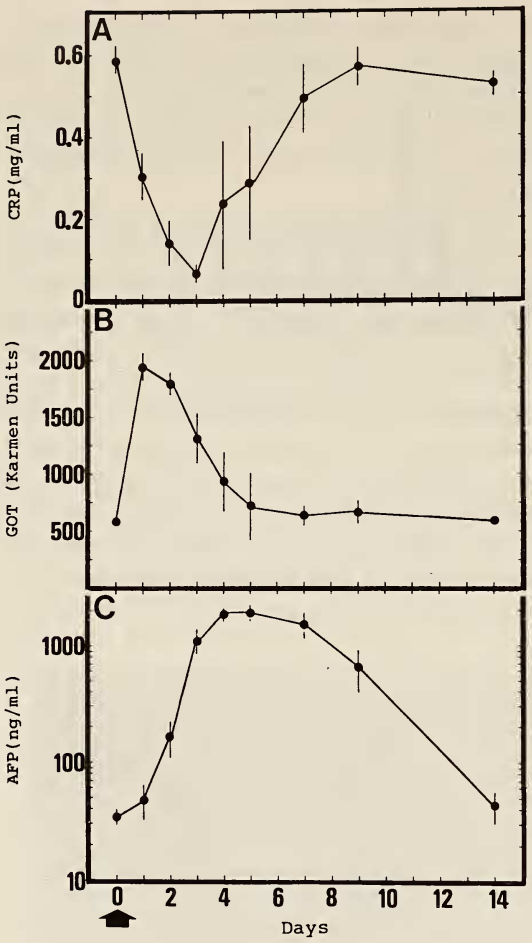

FIG. 9. Changes in serum levels of CRP (A), GOT (B) and AFP (C) in CCl_4 injected rats. CRP was determined by rocket immunoelectrophoresis, GOT by the method of Karmen [92] and AFP level by enzyme-immunoassay [77].

Figure 9 shows the changes of the serum level of CRP, GOT and α-fetoprotein (AFP) in CCl_4-intoxication. CRP markedly decreased to 0.03–0.05 mg/ml within 2 to 3 days and returned to the initial level on day 7. GOT rapidly elevated on the 1st day after injection. The increasing serum AFP level indicates the appearence of regenerating cells in liver [69]. Immunohistochemical staining of CRP revealed that CRP appeared in the nuclei of CCl_4-intoxicated hepatocytes (Fig. 8B). These results confirm that rat CRP actually bind to the nuclei *in vivo*. Furthermore, the CRP in rat plays a role as a scavenger of endogenous material such as nuclei derived from damaged cells [41].

The changes in serum levels of CRP during chemical carcinogenesis were observed. The rats (Donryu strain, male) were fed on a diet containing 0.06% 3′-methyl-4-dimethylaminoazobenzene (3′-MeDAB) for 10 weeks and then the normal diet until sacrifice [70]. CRP level did not change remarkably through the first 8 weeks, but significantly decreased to about half level of normal (0.28 mg/ml) in the 10th week. Then it gradually increased until the 15th week (Fig. 10). Immunohistochemically, CRP was strongly stained in the non-cancerous area but not in the cancer cells [41]. CRP is able to bind to damaged cells but not intact cells including malignant cells except some kinds of lymphocytes. It seems unlikely that CRP itself plays an important role in carcinogenesis by affecting the metabolism of the azo-dye. Onoe *et al.* [70] observed that normal heptocytes gradually dis-

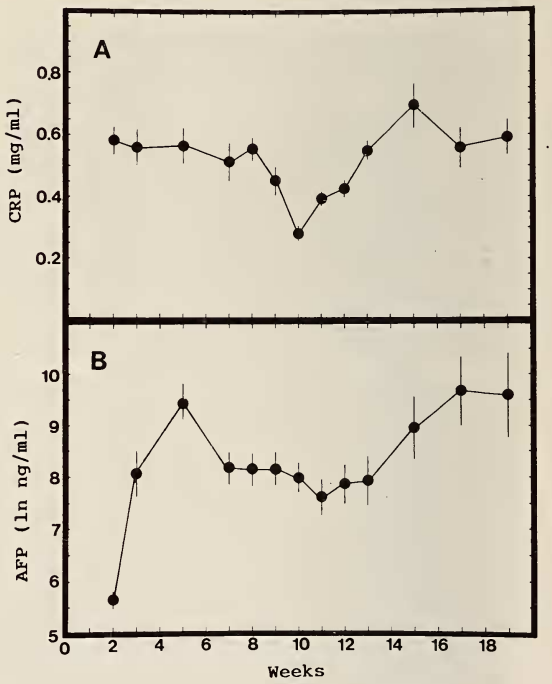

FIG. 10. Changes in serum levels of CRP (A) and AFP (B) during hepatocarcinogenesis. Sixteen rats were fed with diet containing 3′Me-DAB for 10 weeks, followed by normal diet. The CRP was determined by rocket immunoelectrophoresis and AFP by enzymeimmunoassay [77]. Results are expressed as mean±S.E. of 16 rats. Note that AFP level is represented ln ng/ml.

appeared in the first 10 weeks after 3'-MeDAB ingestion, being replaced by regenerating hepatocytes and a new type cells (named "oval cells"). In the present study, the decrease of serum CRP was found after the 9th to 10th week when the liver was occupied by the regenerating cells. Such regenerating cells do not produce CRP. Summerizing these observations, CRP is thought to be produced by normal adult hepatocytes, but neither by regenerating hepatocytes nor cancer cells.

INTERACTION OF CRP WITH MACROPHAGES

Immunohistochemical observation of rat CRP indicates that there may be some interactions between CRP and lymphocytes. Several reports demonstrated the relationship between CRP and macrophages [16–21]. Rat CRP is somewhat different in structure and the serum level from human as described above.

In rat, the interaction of CRP and lymphocytes was investigated *in vitro* and *in vivo*. Although CRP was detected in the culture medium of hepatocytes, the concentration of CRP gradually decreased with time. By adding the culture medium of macrophages obtained from rats elicited by an intraperitoneal injection of glycogen, the sysnthesis of CRP was sustained. However, the culture of thymocytes or splenocytes obtained from rat injected with turpentine-oil, yielded no such effect (Fig. 11). CRP was detected neither in the culture medium of each lymphocyte nor macrophages culture. The effect of the culture medium of macrophages on the serum level of CRP in 10-day-old rats was observed [65]. The serum level of CRP was significantly increased by injecting the culture medium of macrophages compared with rats injected with plane culture medium or no treatment [65]. The synthesis of CRP in heptocytes is initiated by interleukin (IL)-6 produced by macrophages. In preliminary examination, a recombinant human IL-6 strongly enhanced a production of CRP by rat hepatocytes *in vitro*, but not recombinant human IL-1. When ^{125}I labeled recombinant human IL-6 was injected into rat, the radioactivity was localized in hepatocyts. [71]. It was demonstrated that the acute phase

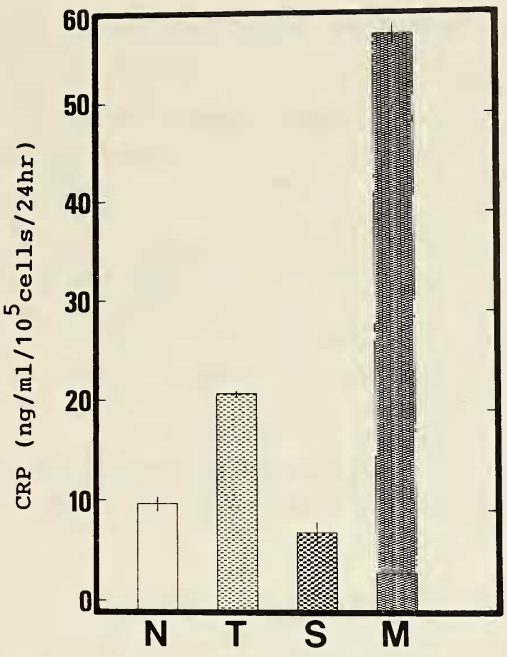

FIG. 11. Effect of culture media of lymphocytes on the production of CRP by the primary culture of rat hepatocytes. N, none (control); T, thymocytes; S, splenocytes and M, macrophages. The hepatocytes were cultured for 54 hr. The concentration of CRP in culture media were determined by enzyme-immunoassay [77]. Thymocytes and splenocytes were obtained from rats 24 hr after njection of turpentine-oil, and macrophages obtained from rats 5 days after an intraperitoneally injection of 5% glycogen. Vertical bars represent S.D.

reactants, for example AGP, α_2-macroglobulin and albumin in rat, were controled by recombinant human IL-6 [72, 73]. However, it has not been known whether CRP in fishes are also controlled by soluble factor(s) derived from macrophages such as IL-6 or not.

Release of superoxide anion from rat macrophages increased by adding CRP to the culture of macrophages [65]. The findings, including immunohistochemically localization of CRP, may suggest that CRP serves as a physiolosical macrophage activator, contributing to the acceleration of a nonspecific host resistance in inflammatory response. In its subunits, human CRP has an analogous structure to "Tuftsin" which is a peptide consisted of four amino acid, THR-LYS-PRO-ARG, and strongly activates macrophages [74,

75]. Although the complete amino acid sequences of rat CRP has not been known, 45 carboxyl terminal residues were determined [61]. The rat CRP also has a Tuftsin analogue sequence, ILE-LYS-PRO-GLN. The results would indicate that CRP may be a source of the macrophage-activating peptide. I propose that CRP locally gathering around the site of inflammation is bound to macrophages, and then by digestive protease(s) of the cells a small peptide such as Tuftsin is derived from subunits of CRP to activate the macrophages. Actually, Robey *et al.* reported that the peptide(s) produced by the proteolysis of human CRP have an immunomodulating activity [76]. There may be a system for amplification by CRP and macrophages in the primary stage of defence.

SEX HORMONES AND CRP

The mean concentration of rat serum CRP was 3.6 ± 0.8 μg/ml (n=5) at the birth and no apparent change was observed during the first 15 days. Thereafter it increased rapidly until day 30 reaching 0.1 mg/ml and further increased gradually to the adult level of 0.4–0.8 mg/ml. No differences were observed between male and female [77]. The change of serum AFP, which is a typical onco-fetal protein in mammals [78], in newborn rats is also shown in Figure 12, decreasing with age in contrast to CRP.

Serum CRP level after conception showed two peaks before deliverly. The first peak was seen on the day of fertilization and the second peak was observed on day 15 of gestation. The mean levels at the peaks were 0.70 ± 0.06 and 0.77 ± 0.10 mg/ml, respectively. Then the CRP decreased markedly to 0.42 ± 0.05 mg/ml on the day of parturition (day 20 of gestation) and elevated again to the normal level within 2 days. The CRP levels on day 0, 15 and 20 significantly were high, as compared to the control ($P<0.05$), examined by the

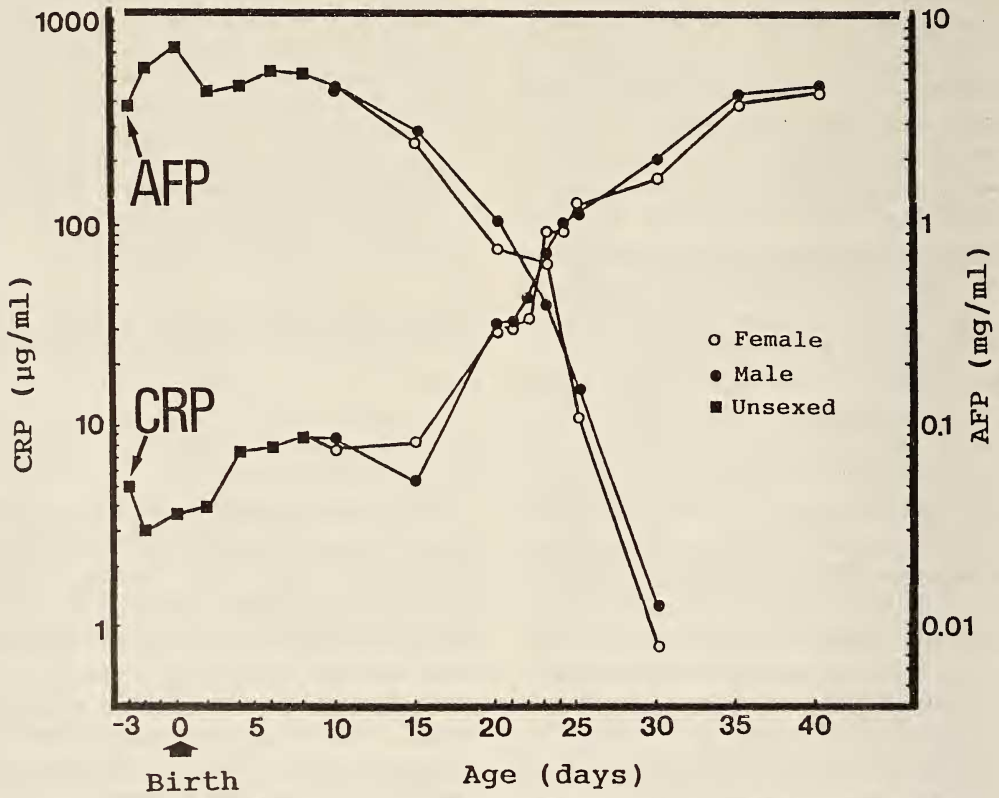

FIG. 12. The changes in serum levels of CRP and AFP during development of rats. Both levels were determiend by rocket immunoelectrophoresis, and represented as the value of 5–10 rats.

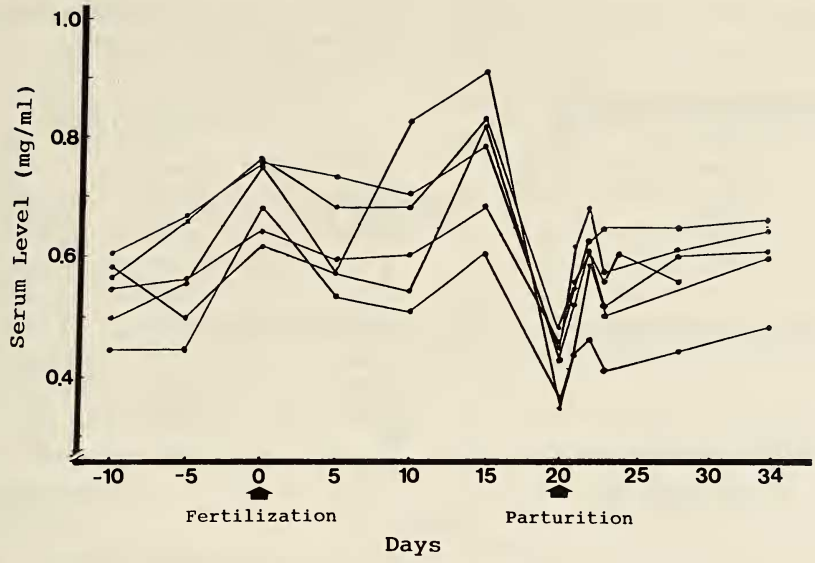

FIG. 13. The changes in serum CRP levels of pregnant rats. The levels of CRP were determined by rocket immunoelectrophoresis.

2-tailed Student *t*-test (Fig. 13).

It is interesting that the CRP level in rats changed with its development, especially, during 10 days after 15-day-old, increasing 60-times. Zouaghi *et al.* [79] reported that in neonatal rats, injection of turpentine-oil caused the decline of serum level of AFP, a well known carrier of estrogen [80], with a marked increase of haptoglobin being one of the acute phase reactants. Although, it is not clear whether the production of CRP relates to that of AFP or not, the serum levels of both proteins may be controlled by estradiol. According to Döhler and Wuttke, the serum level of estrogen in newborn rat is 10 times higher than that of adult [81]. The serum level of estradiol increases towared the parturition [82]. These results suggest that the production of CRP in rat is controled by estradiol. In fact, when a rat was injected with estradiol-17β, the serum level of CRP was decreased. However, no significant effect of other steroid hormones was observed (Fig. 14). In contrast, the serum level of AGP, a

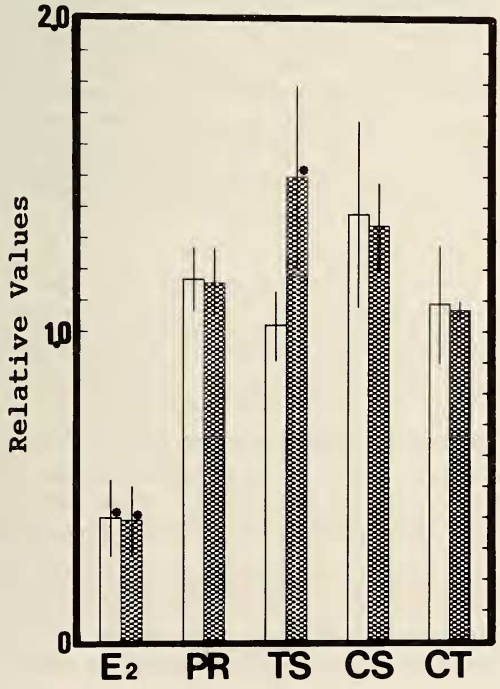

FIG. 14. Effect of steroid hormones on the serum level of CRP in rats. E_2; estradiol-17β, PR; progestrone, TS, testosterone; CS, corticosterone; CT, control. Five rats were dialy (2 days) injected s.c. with a dose of 1 mg/kg in sesame oil or oil alone as contorl. The serum level of CRP was determined by rocket immunoelectrophoresis. The values were represented as the ratio to the initial level (0.57 ± 0.12 mg/ml). Open and meshly horizontal bars indicate intact and ovariectomized rats, respectively. * Statistically significant over the control: $P < 0.05$.

major acute phase protein in rat, was remarkably increased by administration of estradiol [83]. These results indicate that the changes of serum level of CRP might be a hormonal effect but not acute injuries of hepatocytes. In both syrian hamster and white-edged rockfish, CRPs are so-called "female protein". In syrian hamster, the serum level of CRP in female is as high as 1.5–3 mg/ml and that of male is 4–20 μg/ml [84, 85]. The serum level of CRP in male syrian hamster was increased by removing the testis. By injection to female animals of testosterone, the serum level of CRP in female was decreased. The production of CRP was suppressed by testosterone in syrian hamster. On the other hand, the serum level of the CRP analogue in white-edged rockfish was enhanced by estradiol-17β but not testosterone and cortisol. In the male fish, the level was remarkably elevated from the initial level of 25.0 ± 19.0 μg/ml to 1533.3 ± 484.0 μg/ml (n=5) during 10 days after injection with estradiol–17β (1 mg/kg) [33].

Although it is well known that human chorionic gonadotropin (hCG) has an immunosuppressive activity during pregnancy [86], there is no report describing the effect of hCG on the serum level of acute phase reactants. Injection of hCG to normal male rats significantly elevated the serum level of CRP as well as AGP (Table 2). This elevation was independent of the dose commonly used for rat experiments. At present, the mechanism of hormonal control of CRP production in hepatocytes has not been known yet. It is important to investigate carefully the relationship between the sex hormones and CRP synthesis in hepatocytes.

TABLE 2. Serum levels of acute phase reactants in rats injected with hCG

Groups		CRP (mg/ml)	AGP (mg/dl)
Control		0.41 ± 0.01	6.1 ± 0.9
hCG treated			
I	20 IU	0.57 ± 0.03**	9.3 ± 0.6*
II	100 IU	0.63 ± 0.03***	9.8 ± 0.9*
III	300 IU	0.63 ± 0.03***	10.6 ± 0.8**
IV	1000 IU	0.58 ± 0.01***	13.5 ± 1.7**

Mean$\pm$S.E.; P *<0.05, **<0.01, ***<0.001
Serum was collected 24 hr after injection. Serum CRP and AGP levels were determiened by rocket-immunoelectrophoresis and single radial immunodiffusion [93], respectively.

CRP IN STRESS

The topic in recent studies on CRP is a relationship with stress. The serum level of CRP in rainbow trout markedly elevated when fish reared in water of high tempareture [87]. A preliminary experiment in our laboratory, when 8 rats reared in a cage of $29\times34\times16$ cm for 16 days (crwoding stress), the serum level of CRP was slightly increased at Day 3 and then decreased to the initial level on Day 16 (unpublished data). The relationship between CRP and stress has not been studied in detail.

CONCLUSIONS

Since 1930, CRP has been used only as a marker for inflammation in the clinical laboratories, however, a number of studies let us know that this protein has active functions related to the defence system. The biological functions of CRP are summerized in Fig. 15. CRP is synthesized in hepatocytes, stimulated by IL-6 derived from macrophages in inflammatory sites. CRP activates macrophages and inhibit the action of T-cell [88–90] to block production of antibody against endogenous materials. CRP acts as a scavenger of endogenous materials, cooperating with complements. In rat, CRP does not activate complement, C3 [46]. On the other hand, production of CRP is controlled by sex hormone(s).

The studies on CRP have remained a lot of problems to be solved, for example the evolutionary origin of this protein, to act really as a scavenger of endogenous substances in lower classes of animals having undeveloped immune net works, functional relationship with lectin, the activity as a modulator of immune response and relationship with stress. It is neccesary to investigate what role CRP plays in the reproduction. Unfortunately, studies on this protein have been carried out in the field of medicine and veterinary, and few reports have been brought from zoological field. I hope that more investigators will be intersted in CRP and the mechanism of defence system in animals

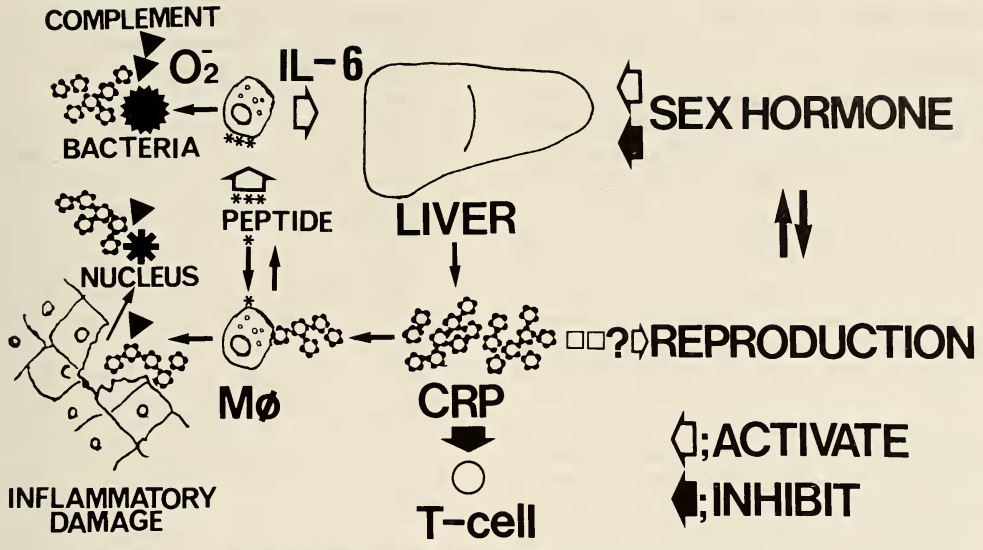

FIG. 15. The mechanism of sysnthesis and the biological functions of CRP.

will be soon clarified.

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